ORIGINAL ARTICLE

Plasma and urinary Endothelin-1 concentrations in asphyxiated newbors

Neslihan Tekin [2], Ener Cagri Dinleyici [1], Mehmet Arif Aksit [2], Nurdan Kural [3] & Kevser Erol [4]

1. Dept. of Pediatrics, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey
2. Division of Neonatology, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey
3. Dept. of Pediatric Nephrology, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey
4. Dept. of Pharmacology, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey

Correspondence to: Ener Cagri Dinleyici, MD.
Department of Pediatrics, Eskisehir Osmangazi University, Faculty of Medicine,
TR-26480 Eskisehir, TURKEY
PHONE/FAX: +90 222 2290064
EMAIL: timboothtr@yahoo.com

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Abstract

The aim of this study was to determine if there is any correlation between the hypoxia induced deterioration of renal functions and urinary excretions of endothelin (ET). Therefore using a sensitive and specific radioimmunoassay, we have investigated plasma ET-1 concentrations and urine ET-1 excretions in healthy and asphyxiated newborns. Sixteen newborns (10 boys, 6 girls) with perinatal asphyxia or hypoxia of variable seriousness which were followed at Newborn Intensive Care Unit in Eskisehir Osmangazi University Faculty of Medicine were enrolled. Simultaneously, gestation and weight matched 10 newborns (6 boys, 4 girls) with no asphyxia (first minute Apgar score >7) were enrolled as controls.
Plasma ET-1 concentrations of the asphyxiated infants (61.8±79.3 pg/ml, between 23.4–125.2 pg/ml) were higher than in the control group (29.3±22.1 pg/ml, between 12.3 and 50.8 pg/ml, $p<0.05$). However creatinine clearance values were not different between the two groups ($p>0.05$), mean fractional excretion of sodium levels (FeNa%) were higher in the study group than the controls ($p<0.01$). Urinary ET-1 concentrations in the asphyxiated infants were 144.6±63.4 pg/ml versus 70.1±27.7 pg/ml in the control group ($p<0.001$). The ET clearance were more elevated in the asphyxiated newborns than in the healthy infants ($p<0.05$). Urinary ET-1/Cr ratio in the hypoxic infants were significantly elevated in the first day of life when compared with those of healthy infants ($p<0.05$). Total ET excretion was negatively correlated with FeNa (%) ($r=-0.603$, $p<0.05$). Plasma ET-1 concentrations of the asphyxiated infants reduced at 48 hours of age ($p<0.001$). Fifth minute Apgar score was negatively correlated with urinary ET-1 levels ($r=-0.615$, $p<0.01$), urinary Na excretion ($r=-0.583$, $p<0.01$), FeNa (%) ($r=-0.597$, $p<0.01$) and total ET excretion ($r=-0.560$, $p<0.01$) and positively correlated with ET clearance ($r=0.559$, $p<0.05$). Urinary ET-1 levels were negatively correlated with umbilical artery BE levels ($r=-0.612$, $p<0.05$).
To our study, elevated urinary ET-1 levels were observed during perinatal asphyxia and urinary ET-1 levels were negatively correlated with 5th minute Apgar score and cord blood base excess levels. For this reason urinary ET-1 levels could be

a marker of perinatal asphyxia as cord blood ET-1 levels. With investigations showing renal production is independent from plasma and increased urinary ET-1/Cr levels in newborn with perinatal asphyixia and also negative correlation between the total ET excretion and FeNa, urinary ET-1 levels could be served as a useful marker to detecting also impaired renal functions in infants with perinatal asphyxia.

INTRODUCTION

Perinatal asphyxia is one of the major causes of neonatal deaths and neurodevelopmental sequel in worldwide despite marked improvement in perinatal care. Asphyxia can cause multi-organ dysfunction and perfusion to more vital organs like heart, brain and adrenals is maintained at the expense of kidneys, gut and skin. As a consequence, kidney is one of the frequently injured organs in newborns with perinatal asphyxia [1,2].

Endothelin (ET) is a powerful vasoconstrictor peptide synthesized and secreted by the vascular endothelium and conflicting results have been reported on endothelin-1 (ET-1) during the neonatal period [3–6]. Elevated levels have been found in various neonatal diseases, such as persistent pulmonary hypertension [7]. Isozaki-Fukuda et al.[8] reported that the perinatal asphyxia play a pivotal role as a triggering for ET-1 synthesis and secretion and Takada et al. [9] reported elevated ET-1 levels during foetal asphyxia in an experimental model.

Huang and Liu [10] reported that urinary ET levels reflect the maturity of kidney in healthy newborn and urinary ET mainly produced in renal cells. Significant amounts of ET are produced by non-endothelial cells, mainly tubular-epithelial and mesengial cells [11]. Large amounts of ET are found in urine compared with the small amount present in blood [12]. Renal ET-1 production is increased by hypoxia and has been implicated in ischemia-induced renal hypoperfusion. The aim of this study was to determine if there is any correlation between the hypoxia induced deterioration of renal functions and urinary excretions of ET. Therefore using a sensitive and specific radioimmunoassay, we have investigated plasma ET-1 concentrations and urine ET-1 excretions in healthy and asphyxiated newborns.

MATERIAL AND METHOD

This was a prospective case-control study conducted in a level III referral neonatal unit. Sixteen newborns (10 boys, 6 girls) with perinatal asphyxia or hypoxia of variable seriousness which were followed at Newborn Intensive Care Unit in Eskisehir Osmangazi University Faculty of Medicine were enrolled. Simultaneously, gestation and weight matched inborn neonates with no asphyxia (first minute Apgar score >7) were enrolled as controls. Control group was consisted of 10 newborns (6 boys, 4 girls). This study includes infants which were 34 weeks gestational age or above. This lower limit was chosen because nephrogenesis is complete by this time and comparisons are not usually complicated by developmentally determined differences in renal function and above 30 weeks urinary ET-1 excretion is not different from term infants.

The infants were identified to have experienced perinatal asphyxia if at least three of the following criteria were present: 5th minute Apgar score <6, metabolic acidosis (serum bicarbonate <12, and/or pH<7.2 in cord blood or in the first hour after birth) and abnormal physical findings, or onset of spontaneous respiration at >5 minutes and/or requirement of more than one minute of positive pressure ventilation before sustained respiration occurred. Newborn infants with congenital malformations, metabolic disorders and other systemic disorders were excluded form the study.

A 24-hour urine was collected and blood samples were obtained by inserting a needle into a hand vein and allowing a free flow of blood into EDTA containing tubes in the first day of life in the both groups. After 48 hours of age the same procedure was repeated only in asphyxiated infants. Each sample was divided into two parts. Serum electrolytes, serum creatinine, blood urea were measured once a day. Creatinine clearance (CrCl) and fractional excretion of sodium (FeNa) were determined. The remaining parts of blood and urine samples were stored at –80 °C until analysis of ET-1. ET-1 was assayed in serum and urine samples using an endothelin-1 specific radioimmunoassay system (Amersham Life Science, RPA 555) following the standard Amersham Protocol.

All statistical analysis was performed using the SPSS 10.0 for Windows software package (IL, Chicago, USA). For the statistical analysis, student-t test parametric test and paired test were used for comparison, Mann Whitney U test was used for correlations. Data were expressed as mean±SD. Differences with $p<0.05$ were considered to be statistically significant.

RESULTS

In the study group, mean gestational age was 38.3±1.9 weeks (34–41 weeks) and mean birth weight was 2 821±1 009 (1 700–4 600 g). Mean gestational age of the control group was 38.8±1.1 weeks (38–41 weeks) and with a mean birth weight as 3 258±415 g (2 800–4 350 g). Mean gestational age and birth weight were similar between the study and the control group ($p>0.05$). Of the 16 infants with asphyxia, 12 were born at term (>37 weeks) with a gestational age of 38.4±2.1 weeks and mean birth weight of 3 167±500 g. Twelve infants had an Apgar score less than 6 at 5 minutes, 11 infants with pH under 7.2 and base excess < –12 mmol/L in the first hour of life. Mean 5th minute Apgar score, pH and base excess were 4.4±1.9, 7.12±0.1 and –13.9±1.9 respectively in asphyxia group and 9.7±0.4, 7.33±0.1 and –3.6±1.6 respectively in control group.

Table 1. Renal functions parameters, plasma and urinary ET-1 levels at first day of life in asphyxia and control group.

	Patient group (n=16)	Controls (n=10)	p-value
BUN (mg/dL)	11.6±4.3	10.4±2.4	ns
Creatinine (mg/dL)	0.91±0.2	0.77±0.24	ns
Serum sodium (mEq/L)	132.2±9.2	136.6±3.0	ns
Plasma ET-1 (pg/mL)	26.3 (23.4–125.2)	22.8 (12.3–50.8)	ns
Urine volume (ml/kg/h)	1.17±0.4	1.02±0.3	ns
Daily urinary output (ml)	48.3±14.7	51.0±17.6	ns
Urinary Cr (mg/dl)	37.0±26.7	61.5±25.6	Ns
Urinary Na (mEq/L)	47.2±30.9	24.7±13.4	**p<0.05**
Urinary K (mEq/L)	27.6±22.0	27.4±14.5	ns
Urinary ET-1 (pg/ml)	144.6±63.4	70.1±27.7	**p<0.001**
Urinary ET-1/Cr (pg/mgCR)	6.47±1.77	2.0±0.4	**p<0.01**
Creatinine clearance (ml/min/kg)	0.45±0.33	0.69±0.4	ns
FeNa (%)	2.10±2.26	0.27±0.24	**p<0.01**
Total ET-1 excretion (ng/kg/day)	2.6±0.5	1.0±0.3	**p<0.05**

Table 2. Renal functions parameters, plasma and urinary ET-1 levels at first day of life in asphyxia at first day and 48th hour of life.

	Patient group First day	Patient group 48th hour	p-value
BUN (mg/dL)	11.6±4.3	12.7±7.4	ns
Creatinine (mg/dL)	0.91±0.2	0.8±0.4	ns
Serum sodium (mEq/L)	132.2±9.2	135.2± 11.1	ns
Plasma ET-1 (pg/mL)	26.3 (23.4–125.2)	17.0 (12.7–21.7)	**p<0.05**
Urine volume (ml/kg/h)	1.17±0.4	1.79±0.2	ns
Daily urinary output (ml)	48.3±14.7	71.0±61.2	**p<0.05**
Urinary Cr (mg/dl)	37.0±26.7	40.1±24.1	**p<0.05**
Urinary Na (mEq/L)	47.2±30.9	38.8±23.9	ns
Urinary K (mEq/L)	27.6±22.0	19.7±12.6	ns
Urinary ET-1 (pg/ml)	144.6±63.4	115.8±60.7	ns
Urinary ET-1/Cr (pg/mgCR)	6.47±1.77	3.8±0.6	ns
Creatinine clearance (ml/min/kg)	0.45±0.33	1.35±1.79	ns
FeNa (%)	2.10±2.26	0.84±0.7	**p<0.05**
Total ET-1 excretion (ng/kg/day)	2.6±0.5	2.5±0.4	ns

* Wilcoxon-Mc Nemar

Serum BUN, creatinine, and sodium levels were not different between the study and control group (p>0.05). Plasma ET-1 concentrations of the asphyxiated infants (61.8±79.3 pg/ml, between 23.4–125.2 pg/ml, 95%CI) were higher than in the control group (29.3±22.1 pg/ml, between 12.3 and 50.8 pg/ml, p<0.05) (Figure 1). However creatinine clearance values were not different between the two groups (p>0.05), mean fractional excretion of sodium levels were higher in the study group than the controls (p<0.01). Urinary sodium levels were higher in asphyxiated group than the control group (p<0.05). Urinary potassium and creatinine excretion were similar between the study and control group (p>0.05). Urinary ET-1 concentrations in the asphyxiated infants were 144.6±63.4 pg/ml versus 70.1±27.7 pg/ml in the control group (p<0.001) (Figure 2). The total ET excretion clearance were elevated in the asphyxiated newborns than in the healthy infants (p<0.01). Urinary ET-1/Cr ratio in the hypoxic infants were significantly elevated in the first day of life when compared with those of healthy infants (p<0.01) (Table 1). Total ET excretion was negatively correlated with FeNa (%) (r=–0.603, p<0.05).

Plasma ET-1 concentrations of the asphyxiated infants reduced at 48 hours of age (p<0.001). The mean value of urinary ET-1 excretion was 118.6±60.7 on the second day in hypoxic group, was not significantly different from the first day mean value (144.6±63.4) (p>0.05). FeNa (%) values were reduced at 48 hour of life (0.84±0.7) however first observed levels was 2.10±2.26 (p<0.05).

Fifth minute Apgar score was negatively correlated with urinary ET-1 levels (r=–0.615, p<0.01), urinary Na excretion (r=–0.583, p<0.01), FeNa (%) (r=–0.597, p<0.01) and ET excretion (r=–0.560, p<0.01) and positively correlated with total ET excretion (r=0.559, p<0.05). Umbilical artery pH and BE levels were negatively correlated only urinary sodium excretion (r=–0.583, p<0.05, r=–0.599, p<0.05 respectively). Urinary ET-1 levels were negatively correlated with umbilical artery BE levels (r=–0.612, p<0.05).

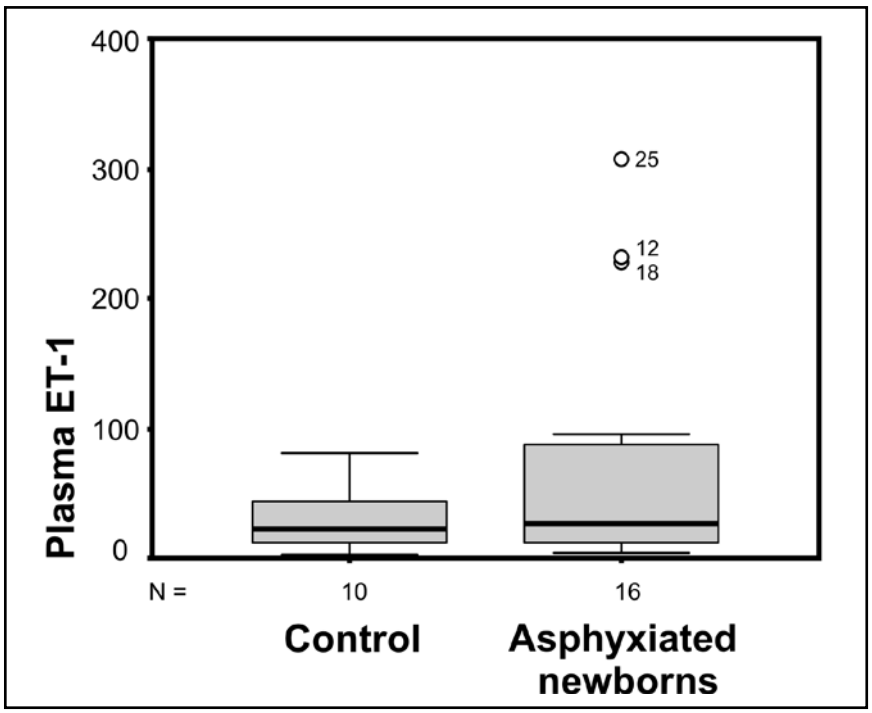

Figure 1. Plasma endothelin-1 levels in asphyxiated and healthy newborns.

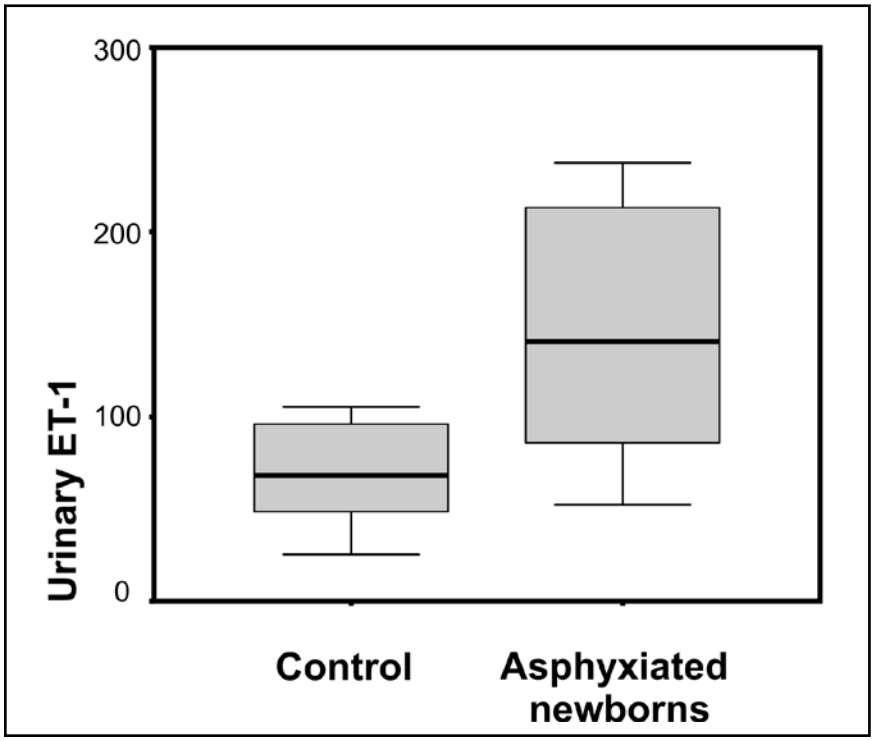

Figure 2. Urinary endothelin-1 levels in asphyxiated and healthy newborns.

CONCLUSION

Kidney is one of the target organs of hypoxia. Moderate to severe renal involvement has been reported up to 42% in newborns infants but subclinical, hypoxic renal damage may be relatively common [13]. In our study, with regard to the values of BUN and plasma creatinine, overt renal insufficiency was not developed in any of the infant with fulfilled criteria for asphyxia. It was suggested that renal ET-1 production was increased by hypoxia in inner medullary collecting duct and has been implicated in ischemia induced renal hypoperfusion. In the kidney ET has significant effects on renovascular, glomerular, and tubular functions, causes severe vasoconstriction resulting in a decrease in renal blood flow and GFR, inhibit sodium reabsorbtion and vasopressin induced water transport [14]. Increase in urinary ET-1 excretion has been found in several forms of renal failure both chronic

and acute, diabetes mellitus, and contrast nephrotoxicity [15]. To our study urinary ET-1 levels were higher in asphyxiated newborns. In the studies – like our study – it was shown that there was not any correlation between urinary ET-1 excretion and its plasma levels. We found elevated urinary ET-1 levels in asphyxiated groups with also increased levels of urinary sodium, ET clearance, and FeNa. In an experimental study, Nir et al. [16] found significant increase urinary ET excretion, urine flow, urinary sodium excretion and fractional excretion of sodium after 60 minutes of asphyxia. Kojima et al. [17] in their study with hypoxic infants determined significantly elevation in fractional excretion of sodium and ET clearance in hypoxic infants compared with the healthy infants. Urinary excretion of ET-1 was significantly higher in asphyxiated newborns. These data supported the investigations showing renal production is independent from plasma. It was also demonstrated that acute moderate hypoxia results in increased urinary ET excretion in association with increase in fractional excretion of sodium suggesting the role of endogenously produced renal ET in the regulation of sodium homeostasis during hypoxia as it was shown in previous studies.

Recently, higher ET-1 levels were found in newborns with low 5'-Apgar score, suggesting that ET-1 could be a marker of perinatal asphyxia [6]. Our results showed that hypoxia tended to elevate the plasma ET-1 levels. On the other hand after 48 hours of age the mean value of plasma ET-1 level significantly reduced in hypoxic infants when compared with first day values probably due to improvement in hypoxic state. Fifth minute Apgar score was negatively correlated with urinary ET-1 levels, urinary Na excretion, FeNa (%), and total ET excretion. While the 5th minute Apgar score could not demonstrate always asphyxia, our study population includes all criteria of the perinatal asphyxia. Although umbilical acid-base status is better indicator for asphyxia and we found negative correlation between urinary ET-1 levels and umbilical cord base excess levels. However we could not demonstrate this relationship between the urinary ET-1 levels and cord blood pH levels, our umbilical cord pH levels of our study population were not significantly decreased.

In our study, creatinine clearance and thus GFR were similar in cases as compared to controls. In asphyxiated groups, FeNa levels were significantly higher than the controls. Recent study suggested that FeNa as 2.5% as the cut-off as this value has correlated well with intrinsic renal failure secondary to hypoxia [1]. To our study, 6 out of 16 asphyxiated newborns have FeNa levels as 2.5 and in asphyxiated group, total ET excretion were negatively correlated with FeNa levels. Also 5th minute Apgar score was negatively correlated with FeNa levels.

To our study, elevated urinary ET-1 levels were observed during perinatal asphyxia and urinary ET-1 levels were negatively correlated with 5th minute Apgar score and cord blood base excess levels. For this reason urinary ET-1 levels could be a marker of perinatal asphyxia as a cord blood ET-1 levels. With investigations showing renal production is independent from plasma, increased urinary ET-1 levels in newborn with perinatal asphyxia and also negative correlation between the total ET excretion and FeNa, urinary ET-1 levels could be served as a useful marker to detecting also impaired renal functions in infants with perinatal asphyxia.

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